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Alternative combinations of tillage practices and crop rotations can foster earthworm density and bioturbation

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ABSTRACT

Earthworms, which contribute to important soil functions, suffer from intensive agriculture. Their response depends among other things on the earthworm ecological group (anecic, endogeic, epigeic) and the combination of the applied farming practices. To advice on methodological adaptations that enhance earthworm-mediated soil functions, effects of different practices on earthworms need to be studied in concert. We investigated the effects of tillage intensity (conventional, reduced, no tillage) and crop rotation diversity (simple = wheat, barley; diverse = wheat, peas, oil seed rape) on earthworm density and community composition in a Swedish long-term experiment. Furthermore, we calculated annual earthworm bioturbation to quantify the effects of farming practices on earthworm functions. Total earthworm densities did not vary between the different tillage intensities, but were on average 58% higher in the diverse than in the simple crop rotation. The pattern was mainly due to the response of the most abundant endogeic earthworms, which were not affected by tillage intensity, but were nearly two times more abundant in the diverse than in the simple crop rotation. Densities of anecic earthworms were 17 times higher under no tillage than conventional tillage. Anecic earthworms also benefitted from a diversified crop rotation, but the response depended on tillage intensity. The level of bioturbation reflected the response of anecic earthworms, and was more than four times higher under no tillage, 549 g dw m^{-2} $year^{-1}$, than under conventional tillage. We conclude that highest earthworm bioturbation is best achieved with no tillage. However, earthworm densities and potentially bioturbation can be increased also by a diversified crop rotation, when reducing tillage intensity is not feasible.

1. Introduction

Earthworms (Annelida, Oligochaeta) are ecosystem engineers (Lavelle et al., 1997), which have a major effect on a range of important soil functions that are vital in agriculture (Blouin et al., 2013; Bertrand et al., 2015). Via their burrowing and casting activities earthworms enhance nutrient mineralization (van Groenigen et al., 2019), litter decomposition (Huang et al., 2020), and soil structure formation (Schon et al., 2017). Their presence in agroecosystems has been shown to aid in pest and disease control (Plaas et al., 2019) and increase yields by on average 25% (van Groenigen et al., 2014).

Agricultural methods can strongly affect earthworms, and earthworm densities in arable fields are generally lower than in pastures and permanent grasslands (Curry, 2004). The detrimental effect of conventional tillage practices such as moldboard and rotary ploughing on earthworms is well documented (Briones and Schmidt, 2017; Chan, 2001; Pelosi et al., 2014; van Capelle et al., 2012). However, how earthworms respond to intensive tillage depends on e.g. soil type, timing of the tillage operation and soil moisture conditions during tillage (Chan, 2001; Pelosi et al., 2014), as well as earthworm species and ecological group (Bouché, 1977). Conventional tillage is especially harmful for litter feeding earthworms, both the surface living epigeic earthworms and the deep-burrowing anecic earthworms (Briones and Schmidt, 2017), as ploughing moves litter to deeper soil layers, and destroys the system of permanent burrows inhabited by the anecics (Briones and Schmidt, 2017; Chan, 2001). Endogeic earthworms, which dwell and feed in upper mineral soil (Bouché, 1977; Lavelle, 1988), are relatively tolerant to intensive tillage, and may even benefit from incorporation of crop residues via ploughing (Chan, 2001). Although less intensive tillage practices exist (e.g. cultivator, chisel plough, direct sowing), which are less damaging for earthworms (Briones and Schmidt, 2017), conventional ploughing remains an important practice in many agricultural systems, such as organic farming (Casagrande et al., 2016), and for the cultivation of certain crops and soil types (Soane et al.,

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2012). For those systems, it is important to explore alternative ways to enhance earthworm densities and alleviate the negative effects of intensive tillage.

Agricultural practices that increase the quantity or quality of organic matter inputs to the soil have been shown to increase earthworm densities (Briones and Schmidt, 2017). Such practices include the use of organic fertilizers (Lapied et al., 2009), the application and incorporation of crop residues (Frazão et al., 2019a), planting especially leguminous cover crops (Roarty et al., 2017; Fiorini et al., 2022), and the use of leys (Jarvis et al., 2017) or legumes (Ashworth et al., 2017; Hubbard et al., 1999; Rodríguez et al., 2020) in crop rotations. These practices could alleviate the detrimental effect of intensive tillage on earthworms via an increase in food resources, and speed up the recovery of earthworm populations after tillage operations. However, in order to verify an alleviating effect of different types of organic matter inputs requires comparing the effects of these practices on earthworms with those of tillage intensity in a complete multifactorial design. Although such studies are few, some examples exist. For example, Melman et al. (2019) and Denier et al. (2022) did not find residue retention or cropping system (conventional, feed or biogas), respectively, to enhance earthworm densities under conventional tillage. On the other hand, Crotty et al. (2016) found that, in comparison to other forage crops, a legume (*Trifolium repens*) buffered the reduction in earthworm densities during the first year after conversion from forage to annual cereals both with conventional and no tillage, although the differences in anecic densities after conversion were not statistically significant between the preceding forage species. We are not aware of studies exploring whether other legume species alleviate the detrimental effect of tillage earthworms, or whether such buffering effect has long-term relevance.

Changes in the absolute and relative densities of earthworm ecological groups due to agricultural management are likely reflected in earthworm mediated soil functions both qualitatively and quantitatively (Pelosi et al., 2014). Especially the reduction of large anecic species due to intensive tillage (Briones and Schmidt, 2017) may drastically impair functions such as soil macropore formation (Pelosi et al., 2017; Krogh et al., 2021) and litter decomposition (Huang et al., 2020). However, studies quantifying the contribution of earthworms to soil functions in differently managed soils remain rare, because it is difficult to disentangle the direct causes for functional changes in agricultural soils. One way to demonstrate the overall functional effect of earthworms is to estimate earthworm bioturbation, i.e. the mass of soil translocated by earthworms in a certain area and time-period. Earthworm bioturbation by differently composed earthworm communities can be calculated for example with the help of species-specific egestion rates (Taylor et al., 2019). However, no study thus far has used this method to compare earthworm bioturbation in fields under different agricultural management.

In this study, we examined how tillage intensity and crop rotation diversity (cereal versus cereal/legume crop rotation) affect total earthworm densities, ecological group densities, community composition and bioturbation, which we use as a proxy for earthworm activity and function in the soil. Our main focus was on whether the effect of tillage intensity and crop rotation on earthworms and bioturbation depend on each other, and whether the earthworm ecological groups respond differently to the different management combinations. We also examined the effect of both management types on certain soil properties known to be meaningful for earthworms to explore the indirect drivers of earthworm community change under agricultural management. We specifically tested two hypotheses:

(1) A reduction in earthworm density due to intensive tillage can be mitigated by including a legume in the crop rotation. The mitigation effect will be less pronounced for tillage sensitive anecic and epigeic earthworms than endogeic earthworms.
(2) Tillage intensity, more than crop rotation, determines total earthworm bioturbation, which is due to the sensitivity of anecic species to intensive tillage and their large contribution to bioturbation.

2. Material and methods

2.1. Site characteristics and experimental design

Sampling was conducted between 12th and 14th June 2017 at the Säby experimental site in Uppsala, eastern Sweden (59°49′58″N 17°42′19″E). The sampled long-term experiment was established in 2007 and compares tillage methods of different intensities in two crop rotations. The climate of the region is humid continental with an annual mean air temperature of 6.7 °C and an annual mean precipitation of 547 mm during the past thirty years (Swedish University of Agricultural Sciences, Ultuna weather station, 1988–2017). During the sampling, the average temperature was 14.7 °C, and the mean rainfall was 1.1 mm day^{-1}. The soil at Säby is classified as Eutric Cambisol (Etana et al., 2009) and the soil texture is 23.3% clay, 52.2% silt, 24.5% sand (Arvidsson, 2010). The organic matter content of the soil at the start of the experiment was 4.0% (Arvidsson, 2010). Soil pH in the 30 cm topsoil, averaged over the sampled plots at the experimental site, is 5.56 (SD, standard deviation: 0.33).

The general experimental design is a split plot design with two crop rotations (simple, diverse) as main factors and tillage treatments as sub factors. Three tillage treatments were included in the study: conventional tillage (CT = moldboard ploughing, 23 cm depth), reduced tillage (RT = cultivator, 10–12 cm depth) and no tillage (NT = direct sowing). The simple crop rotation consists of winter wheat (*Triticum aestivum* L.) and spring barley (*Hordeum vulgare* L.), while the diverse crop rotation consists of winter wheat, peas (*Pisum sativum* L.) and oilseed rape (*Brassica napus* L.; Table 1; Supplementary Table S1). The combinations of the crop rotation and tillage treatments are replicated in three blocks with 9 × 21 m large plots (Supplementary Fig. S1). For all crops, residues were left in the field after harvest, and incorporated in the soil in the RT and CT treatments prior to seeding. During the sampling in 2017, i.e. 10 years after the start of the experiment, the crop in both crop rotations was winter wheat. The preceding crop (2016) in the diverse rotation had been peas, while in the simple rotation it was spring barley. Specific amounts of fertilizers and pesticides have been used for different crop species. On average, since the establishment of the experiment, the level of added N and S has been slightly lower and P and K slightly higher in the diverse than in the simple crop rotation. An overview of the applied fertilizers and pesticides in both rotations from 2007 to 2017 is presented in Supplementary Table S1.

2.2. Earthworm sampling

Four samples were taken per plot in the diverse crop rotation and, because of time constraints, two samples per plot in the simple crop rotation. For each sample, a hole of 30 cm (width) x 30 cm (length) x 20

Table 1
Annual crop species in the simple and the diverse crop rotation since the establishment of the experiment in 2007 until the sampling year 2017.

Year	Diverse rotation (DR)	Simple rotation (SR)
2007	Winter wheat	Winter wheat
2008	Peas	Barley
2009	Winter wheat	Winter wheat
2010	Spring oilseed rape	Barley
2011	Winter wheat	Winter wheat
2012	Winter wheat	Winter wheat
2013	Winter wheat	Winter wheat
2014	Spring oilseed rape	Spring barley
2015	Winter wheat	Winter wheat
2016	Peas	Spring barley
2017	Winter wheat	Winter wheat

cm (depth) was dug. The soil collected from the hole was immediately hand-sorted for earthworms. After this, 2.5 l of allyl isothiocyanate (AITC) solution, prepared according to the protocol by Zaborski (2003; 100 mg AITC per 1 l water), was poured in the hole, in order to collect worms deeper in the soil. Each hole was observed for protruding earthworms for 30 min. Collected individuals were rinsed on site in tap water and preserved in 99% ethanol.

The developmental stage of each earthworm was noted and all adult earthworms (indicated by the presence of a clitellum) were identified to species level (Sherlock, 2012). Biomass of the individual worms (g fresh weight, including gut content) was determined by weighing the worms after they had been rinsed in water for 5 min to remove the ethanol and gently dried with a tissue. The biomass was converted from fresh weight to dry weight assuming a water content of 80%, which was considered a realistic average for well-hydrated earthworms (Grant, 1955; Bayley et al., 2010). Adult worms were assigned to main earthworm ecological groups (epigeics, endogeics and anecics) based on Bouché (1977) and Bottinelli et al. (2020). For species representing intermediate ecological groups, such as *Allolobophora chlorotica* (epi-endogeic/intermediate) and *Lumbricus terrestris* (epi-anecic), the main category was used for simplicity (endogeic and anecic, respectively). Juveniles could only be assigned to either the genera *Allolobophora/Aporrectodea* or *Lumbricus*. Thus, percentages of adult individuals in the corresponding genera belonging to the different ecological groups in the complete dataset were used to assign juveniles to the different ecological groups accordingly. Total abundances and biomasses per sample were converted to densities and biomasses per square meter. All samples included severed parts of earthworms, which were not considered in the densities, as they could not be determined to species or converted to numbers of individuals. Thus, the data slightly underestimates natural community densities. How we handled the part biomass data is described in the following section.

2.3. Bioturbation

Average annual bioturbation in 2017, defined as the estimated dry weight of soil translocated via earthworm egestion per square meter, was calculated for each crop rotation/tillage treatment combination using the methodology described in Taylor et al. (2019). In short, the daily egestion rates (g dw faeces g^{-1} body dw day^{-1}) for the different ecological groups, determined in a laboratory experiment by Taylor and Taylor (2014) at 15 °C, were multiplied by the biomass of each earthworm ecological group in one quadrat meter and summed up to total bioturbation (g dw m^{-2} day^{-1}). It was not ideal to include the biomass of earthworm parts in the bioturbation calculation as it was not possible to determine parts to species or ecological groups to assign the egestion rates. However, there was some variation in the biomass of earthworm parts between the treatment combinations (4–41% of the biomass of the whole individuals). Therefore, bioturbation values were calculated twice for each sample, both excluding and including the part biomass, to account for possible discrepancies that could affect the results of the statistical analysis. For the latter values, the part biomass was divided into the different ecological groups based on the proportions of adult earthworm biomass in the respective groups. To calculate bioturbation for a full year, and to account for variation in earthworm egestion in response to temperature, bioturbation at 15 °C was adjusted to field temperatures during the sampling year assuming similar temperature dependency for egestion as for earthworm growth (Taylor and Taylor, 2014). For the field temperatures, we used mean monthly soil temperatures recorded at 10 cm depth at the SLU weather station at Ultuna, which is located approximately 3 km from the experimental site.

2.4. Soil parameters

To contribute to the discussion about the drivers of tillage and crop rotation effects on earthworms, we determined the variation of soil organic carbon (SOC) content, bulk density and water content in the different tillage/crop rotation treatment combinations. Two 30 cm soil cores were collected in each plot with a soil corer of 5 cm diameter, one for SOC and the other for bulk density and soil water content. Each core was divided into three pieces, representing the soil depths 0–10 cm, 10–20 cm and 20–30 cm, to examine differences in the three parameters between the soil depths. The samples were stored at 5 °C until processed. Total carbon content of the samples was determined using the dry combustion method (Elementar Vario El, Heraeus, Hanau, Germany). No inorganic carbon was detected after treatment with HCl, which means that total carbon content in the samples equals organic carbon content (Chatterjee et al., 2009). To determine soil bulk density and water content, the field moist soil samples were weighed, dried at 105 °C for 24 h, and re-weighed.

2.5. Statistical analyses

All statistical analyses were performed in R (version 3.5.3; R core team, 2019). To examine the effects of tillage intensity and crop rotation diversity on earthworm densities, and to determine whether the effect of one type of practice depended on the other, we used generalized linear mixed models (GLMM) from the package *glmmTMB* (Brooks et al., 2017). Models with a similar structure, with tillage intensity, crop rotation diversity and their interaction as explanatory variables, were used to test the effects of these factors on total, anecic and endogeic earthworm densities. Epigeic earthworms were collected in very low densities and were thus left out of the analyses. Three random factors were included in the models: replicate, and the interaction of replicate and crop rotation due to the nested experimental design, and the interaction of replicate, crop rotation and tillage (plot level) due to the pseudoreplication within plots. All models were checked for overdispersion and zero-inflation with the functions testDispersion and testZeroInflation from the *DHARMa* package (Hartig, 2020), and Poisson or negative binomial distribution, and zero-inflated model was applied accordingly (Supplementary Table S2). The significance levels of the effects were determined using the type III ANOVA in the package *car*, with contr.sum contrasts (Fox and Weisberg, 2019). Interaction terms with $P > 0.1$ were excluded from the final model. When an explanatory variable with more than two treatment levels had a significant effect, the differences between specific treatments were further analyzed with Tukey's HSD (Honest Significant Difference) post hoc test using the package *emmeans* (Lenth, 2020). *P*-values smaller than 0.05 are discussed as significant.

Differences in earthworm community composition were explored using multivariate methods in the *vegan* package (Oksanen et al., 2019). To test whether tillage intensity and crop rotation diversity separately or in interdependence affect earthworm community composition, a permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations and marginal effects of terms was performed, using the adonis2 function. We further examined community differences at the sample level by performing a non-metric multidimensional scaling (NMDS) ordination with Bray-Curtis dissimilarity, square root transformation and Wisconsin double standardization, using the package *metaMDS*. Good fit (stress = 0.05) was achieved with four dimensions. Tillage intensity and crop rotations were fitted onto the NMDS ordination using the envfit function, and when the treatment was significantly correlated with the NMDS axes, the different treatment levels were visualized as convex hulls around the sites (Fig. 1). Similarly, we further illustrated the responses of the earthworm ecological groups using the envfit function for the grouped earthworm densities, and projecting the vectors, that were significantly correlated with the NMDS axes (only anecics, see Section 3.3), as arrows on the NMDS diagram (Fig. 1). Earthworm juveniles belonging to the genera *Lumbricus* or either *Allolobophora/Aporrectodea* were treated similarly as separate species in both PERMANOVA and the NMDS.

To test whether total earthworm bioturbation (with and without the

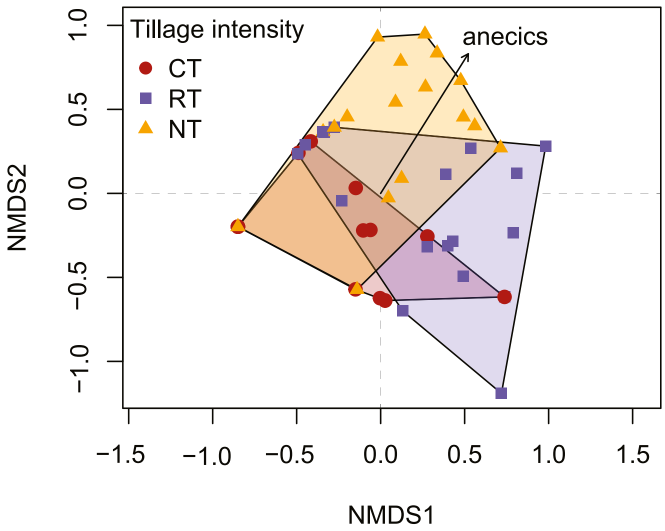

Fig. 1. The two first axes of a non-metric multidimensional scaling (NMDS) ordination presenting differences in earthworm community structure between samples (k = 4, stress = 0.05). Symbols of different shapes and colors and the associated polygons represent samples taken from the plots with different tillage treatments (CT = conventional tillage, RT = reduced tillage, NT = no tillage). Increasing anecic earthworm density is visualized with an arrow, as it was significantly correlated with the NMDS axes.

part biomass), SOC content, soil bulk density and soil water content varied between the tillage and the crop rotation treatments, we used general linear mixed models (LMM) in the package *lme4* (Bates et al., 2015). The explanatory and random variables were the same as for the earthworm models, except that for the soil parameters, we also included interactions of the main treatments and soil depth as an explanatory variable, to test whether the effects of the treatments varied between soil depths. Total bioturbation was ln-transformed and bulk density square root transformed to achieve linearity. Normal distribution and homoscedasticity of the residuals were graphically verified. Same procedure as for the earthworm models was used for determining the significance of the treatment effects as well as for testing differences between treatment levels. Tukey's HSD was also used to evaluate treatment effects on soil parameters averaged over the soil depths. The detailed structure of the models is presented in Supplementary Table S2.

3. Results

3.1. Earthworm densities and community composition

In total, we sampled 443 earthworm individuals, belonging to seven species, of which 90 were adults. The numbers of individuals per sample varied from one to 26, which corresponds to 11–286 individuals (ind.) m^{-2}. The majority of the collected earthworms belonged to the endogeic group (73.8%). Anecic earthworms occurred in intermediate numbers (24.0%), while epigeic earthworms were scarce (2.2%). The most common species was the endogeic *Allolobophora chlorotica* (Savigny, 1826), which in the adult stage was present in 11 of the 18 plots, with densities varying from 11 to 44 ind. m^{-2}. The six other species found were the endogeics *Aporrectodea caliginosa* (Savigny, 1826), *Aporrectodea rosea* (Savigny, 1826), and *Aporrectodea tuberculata* (Eisen, 1874), the epigeic *Lumbricus castaneus* (Savigny, 1826) and the anecics *Lumbricus terrestris* (Linnaeus, 1758) and *Aporrectodea longa* (Ude, 1885). Average densities and standard deviations of all species in the different treatments are presented in Supplementary Table S2.

3.2. Effect of tillage intensity and crop rotation on earthworm densities

The estimated marginal means (EMM) for the total earthworm densities ranged from 51 ind. m^{-2} under conventional tillage in the simple crop rotation to 124 ind. m^{-2} under no tillage in the diverse crop rotation. Total earthworm densities did not significantly differ between the tillage treatments, although there was an apparent increase in total earthworm density with reduced tillage intensity (Fig. 2A). Instead, total earthworm densities were on average 58% higher in the diverse than in the simple crop rotation (Fig. 2A; Table 2). The effect of crop rotation did not depend on tillage intensity.

Endogeic and anecic earthworms responded differently to tillage intensity and crop rotation. Estimated marginal means for endogeic earthworm densities ranged from 43 ind. m^{-2} under conventional tillage in the simple crop rotation to 85 ind. m^{-2} under no tillage in the diverse crop rotation. Endogeic earthworm densities were on average 71% higher in the diverse crop rotation than in the simple crop rotation, and the difference was significant (Fig. 2B; Table 2). Tillage had no effect on endogeic earthworm densities, and the effect of crop rotation did not depend on tillage intensity (Fig. 2B; Table 2).

Anecic earthworms responded significantly to both tillage intensity and crop rotation, and there was a significant interaction of the effects of the two factors (Table 2). More anecic earthworms were found in plots under no tillage than conventional tillage, regardless of the crop rotation (Fig. 2C). Under conventional tillage, anecic earthworm densities were marginally higher in the diverse than in the simple crop rotation (EMM: 4.98 and 0.34 ind. m^{-2}, respectively; $t_{43} = 2.97$, $P = 0.051$). However, there were no significant differences in anecic earthworm densities between the diverse and the simple crop rotation under no tillage (EMM: 43.2 and 54.1 ind. m^{-2}, respectively) and reduced tillage (EMM: 24.0 and 5.82 ind. m^{-2}, respectively).

3.3. Effects of tillage intensity and crop rotation on earthworm community composition

According to the PERMANOVA, both tillage intensity and crop rotation had a significant effect on earthworm community composition (Table 2). The effects of tillage and crop rotation were not interdependent, so the interaction term was not included in the final model. Tillage explained more of the variation than crop rotation ($R^2 = 0.15$ and $R^2 = 0.05$, respectively). Fig. 1 shows differences in earthworm community composition between samples as the two first axes of the NMDS ordination (k = 4, stress = 0.05). Tillage intensity showed significant correlation with the NMDS axes, so the tillage intensity associated with each sample was visualized with the shape and color of the sample point, and convex hulls were drawn around the sample points with the same tillage intensity applied. Similarly, anecic, but not endogeic, earthworm density was significantly correlated with the NMDS axes. This correlation is visualized with an arrow in the NMDS diagram that points in the direction of higher anecic earthworm density in the samples (Fig. 1). Even though the convex hulls largely overlap, the communities under no tillage appear distinct from those of the two tillage treatments. This seems to derive largely from the higher densities of anecic earthworms under no tillage, a pattern also supported by the GLMM results (Table 2).

3.4. Earthworm bioturbation

The models including and excluding biomass of earthworm parts yielded similar results. Thus, and for comparability to the density analyses, only the results for the models excluding the part biomass are presented here. The results including the part biomass are presented in Supplementary Fig. S2. The estimated marginal means for total bioturbation by earthworms from all ecological groups (excluding the part biomass) ranged from 98.0 g dw m^{-2} year^{-1} in the plots under conventional tillage in the simple crop rotation to 742.2 g dw m^{-2} year^{-1} in the plots under no tillage in the simple crop rotation (Fig. 3). Increasing

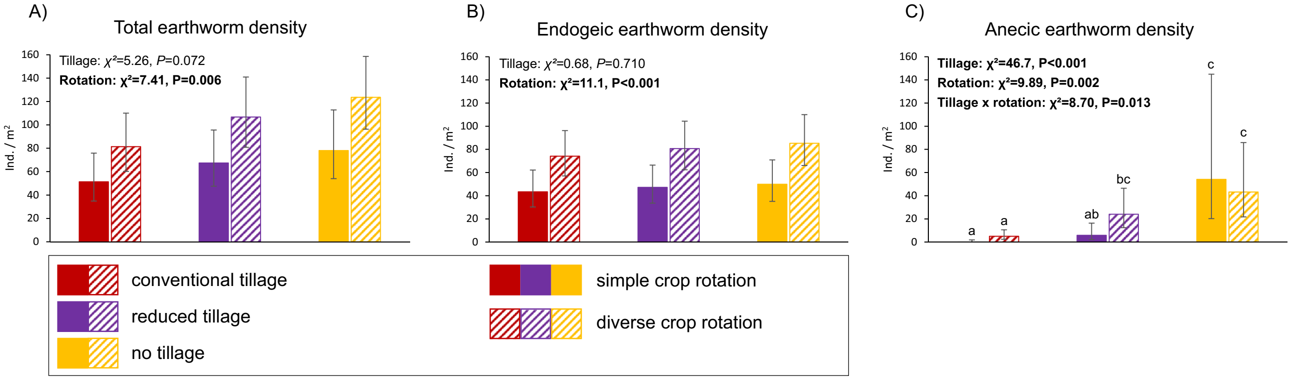

Fig. 2. Estimated marginal means for A) total earthworm densities and densities of B) endogeic and C) anecic earthworms in the different treatments with 95% confidence intervals. For anecic earthworms (C), columns sharing the same letters are not significantly different (Tukey's HSD test, $P > 0.05$). χ^2 and P values for the explanatory variables are presented, and the statistically significant ($P < 0.05$) variables are in **bold** font.

Table 2

Results of the generalized linear mixed models (GLMM), the general linear models (LM), and the permutational multivariate analysis of variance (PERMANOVA) evaluating the effect of tillage intensity, crop rotation and their interaction on endogeic, anecic and total earthworm density, total bioturbation, and earthworm community composition, respectively. Interaction term was only included in the final model when the *P*-value was smaller than 0.1 as in the cases of anecic earthworm density and total bioturbation. *P*-values with $P < 0.05$ are in **bold**.

	Tillage			Crop rotation			Tillage × crop rotation		
Earthworm density (ind./m²)	χ^2	*df*	*P*	χ^2	*df*	*P*	χ^2	*df*	*P*
Endogeic	0.73	2	0.694	6.84	1	**<0.009**			
Anecic	46.7	2	**<0.001**	9.89	1	**0.002**	8.70	2	**0.013**
Total	5.26	2	0.072	7.41	1	**0.006**			
	F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
Total earthworm bioturbation (g dw/m²/yr)	5.56	2	**0.026**	1.40	1	0.349	3.35	2	0.081
	Pseudo-F	*df*	*P*	*Pseudo-F*	*df*	*P*			
Earthworm community composition	4.60	2	**<0.001**	3.24	1	**0.010**			

tillage intensity significantly reduced total bioturbation (Table 2). There were no differences in bioturbation between the crop rotations, and the effect of tillage did not significantly depend on crop rotation. When the interaction term was excluded from the model, the Tukey's HSD post hoc comparison revealed significantly higher bioturbation under no tillage than under conventional tillage ($t_{9.21} = 3.31$, $P = 0.022$).

Endogeic earthworms accounted for most of the bioturbation in the conventionally tilled plots in both crop rotations (99.4% and 97.0% in the simple and the diverse crop rotation, respectively) and in plots with reduced tillage in the simple crop rotation (94.9%). In plots with reduced tillage in the diverse rotation and plots with no tillage in the diverse and the simple crop rotation, the endogeic contribution was lower (58.2%, 32.2% and 25.5%, respectively). In anecic earthworms this pattern was reversed. Their contribution to total bioturbation was very low in conventionally tilled plots in both crop rotations (simple: 0.6%, diverse: 2.8%) and plots with reduced tillage in the simple rotation (4.6%). In plots with reduced tillage in the diverse rotation and no tillage in both crop rotations, the proportion of bioturbation carried out by anecic earthworms was much higher, accounting for 41.4 to 71.3% of the total bioturbation. Epigeics were estimated to contribute little to total bioturbation, values varying between 0.0% in conventionally tilled plots in the simple crop rotation and 3.0% in plots with no tillage in the simple crop rotation.

3.5. *Soil parameters*

Overall, average soil organic carbon content in the top 30 cm did not differ between the different tillage and crop rotation treatments. However, there were differences in soil organic carbon content between the different depths depending on the tillage treatment (Table 3; Supplementary Fig. S3). Typically, under conventional tillage, organic carbon content was evenly distributed throughout the soil profile, whereas under reduced and especially under no tillage, organic carbon content was high in the topsoil and was reduced in the deeper soil. There were no significant differences in soil organic carbon levels in the different soil depths between the two crop rotations.

Average soil bulk density and water content did not vary between the tillage and crop rotation treatments. Expectedly, both soil bulk density and water content increased with soil depth (Table 3), and this pattern did not depend on tillage or crop rotation.

4. Discussion

Our results from the multifactorial long-term experiment provide new evidence that a diversified crop rotation, which includes a legume, can alleviate the detrimental effect of conventional tillage on earthworms, and increase total and endogeic earthworm densities regardless of tillage intensity. Even though anecic earthworm densities were low under conventional tillage, we found that also their densities, and thus potentially bioturbation, can be increased by including a legume in the

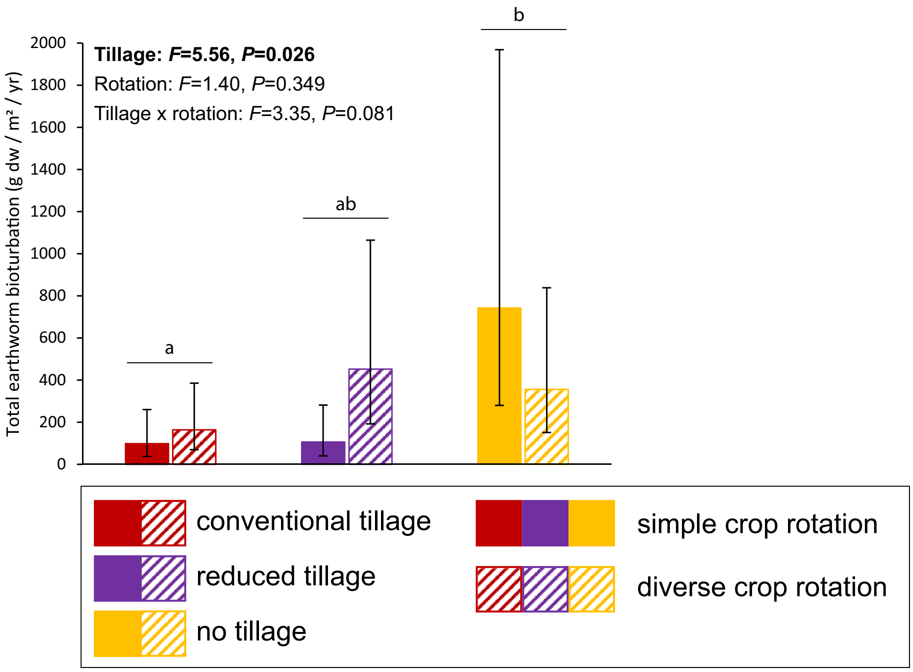

Fig. 3. Estimated marginal means for total bioturbation (g dw / m^2 / yr) in the different treatment combinations with 95% confidence intervals. Columns sharing the same letters are not significantly different (Tukey's HSD test, $P > 0.05$). χ^2 and P values for the explanatory variables are presented, and the statistically significant ($P < 0.05$) variables are in **bold** font.

Table 3

Results of the general linear models (LM) evaluating the effect of tillage intensity, crop rotation, soil depth and the interaction of tillage and soil depth on soil organic carbon content (SOC), soil moisture and bulk density. Interaction term was only included in the final model when the P-value was smaller than 0.1 as in the case of soil organic carbon. *P*-values with $P < 0.05$ are in **bold**.

	Tillage			Crop rotation			Soil depth			Tillage x soil depth		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
SOC	15.5	2	**<0.001**	0.002	1	0.967	0.57	2	0.572	13.0	4	**<0.001**
Soil moisture	2.56	2	0.127	4.10	1	0.180	6.22	2	**0.005**			
Bulk density	1.17	2	0.348	0.088	1	0.795	30.2	2	**<0.001**			

crop rotation. Even small increases in species densities can be meaningful for sustaining the species in the landscape, although restoring more functionally important anecic earthworm densities and bioturbation may require refraining from intensive tillage to allow population recovery.

4.1. Crop rotation matters more for endogeic earthworms than tillage intensity

Endogeic earthworms are generally considered to better tolerate intensive tillage than anecic and epigeic species (e.g. Chan, 2001; Briones and Schmidt, 2017). A meta-analysis by Briones and Schmidt (2017) showed that although endogeic earthworms responded negatively to conventional tillage, they were less affected than anecic and epigeic species. However, the response of endogeic earthworms to intensive tillage varies considerably between individual studies. The majority of studies, including ours, show similar densities of endogeic species in intensively tilled fields compared to fields with reduced or no tillage (Nuutinen, 1992; Pelosi et al., 2014), but some show lower densities (van Capelle et al., 2012), and some higher densities in intensively tilled fields (Baldivieso-Freitas et al., 2017; Boström, 1995). Some of this variation is likely due to species-specific responses, i.e. the composition of the studied earthworm community, and timing of sampling after the tillage event. For example, De Oliveira et al. (2012) found that densities of the endogeic species *Aporrectodea caliginosa* were more strongly reduced by conventional ploughing than those of the endogeic species *Aporrectodea rosea* immediately after tillage, but that the densities of both species recovered in 5–9 months. It has also been speculated that the response of endogeic earthworms to intensive tillage depends on conditions such as soil moisture during tillage, as many endogeic species can become inactive in deeper soil during dry periods, and should then be less affected by tillage (Faber et al., 2017). Different life history traits between species may also explain better tolerance and/or faster recovery of endogeic earthworms after soil disturbance but this topic remains little studied (De Lange et al., 2013). In addition, tillage may have indirect effects on endogeic earthworms via its effects on various soil properties. For example, endogeic earthworms are considered especially sensitive to an increase in soil bulk density (Capowiez et al., 2021) and a decline in organic matter content (Hoeffner et al., 2021), both of which are associated with tillage practices (Blanco-Canqui and Ruis, 2018).

In our study, the earthworm species composition was similar to and the average total densities were comparable to other studies investigating earthworm communities in agricultural soils in the same area (Lagerlöf et al., 2002, 2012). The most common species among adult individuals was *Allolobophora chlorotica.* This species is commonly classified as intermediate or epi-endogeic (Bottinelli et al., 2020): it does not create permanent burrows (Capowiez, 2000), and, although flexible in foraging depth, feeds mainly close to the soil surface (Le Couteulx et al., 2015). There is experimental evidence that, although *A. chlorotica* juveniles may benefit from *L. terrestris* middens for nutrition and burrows for movement (Lowe and Butt, 2007; Uvarov, 2009), *A. chlorotica* also competes with *Lumbricus* species for litter resources, *L. rubellus* often being the strongest competitor (Lowe and Butt, 2002). Simultaneously, *A. chlorotica* and *A. caliginosa* are also considered competitors (Uvarov, 2009). We found a large number of adult *A. chlorotica* under conventional and reduced tillage, but very few under no tillage, where L. *terrestris, Lumbricus* juveniles and the endogeic species *A. rosea* and *A. caliginosa* were more common. Our results match the experimental evidence for the patterns in interactions between these species. Furthermore, Lagerlöf et al. (2002) observed similar complementary density pattern between *A. chlorotica* and *A. rosea* in cultivated fields and their boundaries with different species dominating the two types of habitats in spring compared to autumn. We suggest that under no tillage, with more litter on the soil surface, *Lumbricus* species are better competitors and suppress the numbers of adult *A. chlorotica*. This may further release niche space for endogeic species, as reflected in higher numbers of *A. rosea* and *A. caliginosa*. However, we found many juveniles of both *Lumbricus* and *Aporrectodea/Allolobophora* under no tillage, which cannot be determined to species level using morphological features. Resolving whether the juvenile densities reflect that of the adults and verifying the complementary occurrences of the aforementioned species would require species determination using molecular methods such as DNA barcoding (Maggia et al., 2021).

Tillage can have both short-term and long-term effects on soil organic matter. In the short term, incorporation of crop residues via tillage, especially when grassland is converted to cultivated land (Wyss and Glasstetter, 1992), can strongly increase soil organic matter content. This increase in earthworm food resources in upper soil layers, that are easily accessible for endogeic earthworms, can greatly enhance endogeic earthworm densities (Boström, 1995). In the long term, intensive tillage reduces soil organic carbon (SOC, indicating soil organic matter) content in the upper soil (Meurer et al., 2018), which is likely to reduce endogeic earthworm densities (Hoeffner et al., 2021). In our study, we found differences in the depth distribution of SOC in the upmost 30 cm, with SOC content decreasing with increasing depth in no and reduced tillage and an even distribution of SOC down to 30 cm in conventional tillage. Higher SOC content in the top soil under no tillage may have been of importance to the surface feeding anecic earthworms. The average SOC content down to 30 cm was similar between the different tillage treatments. Frazão and colleagues found no response of endogeic earthworms to crop residue placement in a microcosm study (Frazão et al., 2019b), or to surface application versus incorporation of crop residues in the field (Frazão et al., 2019a). This suggest, that for endogeic earthworms, which move freely through the upper mineral soil, only the total soil organic matter content is important for population densities and not where SOC is located. Our finding of similar endogeic densities and the average SOC contents down to 30 cm in the different tillage treatments is consistent with this idea.

We showed that in the diverse crop rotation, which included peas, oil seed rape, wheat and barley, endogeic earthworm densities were on average more than 70% higher than in the crop rotation including only the two cereals. Several studies have found that crop rotations that include legumes, such as pea, soybean or white clover, enhance earthworm densities compared to continuous monocropping, and rotations with cereals only (Hubbard et al., 1999; Rodríguez et al., 2020; Schmidt et al., 2003). As legume residues serve as an easily palatable resource with high nitrogen content, it has been suggested that it is often the quality rather than the quantity of organic matter as a food resource that limits earthworm densities in arable soils (Curry, 2004). There is also evidence that increased inputs of high quality food resources such as manure (Simonsen et al., 2010) and legume residues (Ashworth et al., 2017), are especially beneficial for endogeic earthworms. In addition to our study, Crotty et al. (2016), Melman et al. (2019) and Denier et al. (2022) compared tillage to another type of agricultural management in a multifactorial experiment. Crotty et al. (2016) found that in comparison to other, mainly non-leguminous preceding forage species, only white clover alleviated the detrimental effect of conventional ploughing on all earthworm ecological groups. The latter two studies did not find residue retention (Melman et al., 2019) or cropping system (conventional, feed, biogas; Denier et al., 2022) to alleviate the detrimental effect of intensive tillage on earthworms. However, in the first case, the only crop species was corn (*Zea mays*), and in the latter, crop rotations in all systems included several legume species. Thus, in these studies, quality of the organic inputs may not have differed enough between the treatments for endogeic densities to diverge.

The best strategy to enhance endogeic and thus total earthworm densities is likely to both improve the quality and increase the quantity and continuous availability of their food resources. Earthworms seem to benefit especially from a crop rotation where crop species with low and high C/N ratio, such as legumes and grasses, alternate (Schmidt et al., 2003; Rodríguez et al., 2020). The benefit of such mixtures is assumed to be due to a combination of a good quality but fast decomposing and thus short term resource (legume), and a lower quality but slower decomposing and thus more continuously available source of nutrition (grass) (Rodríguez et al., 2020). In our long-term experiment, residue biomasses of the different crop species were not measured. Thus, we cannot determine whether quantity or continuity of residues also plays a role in driving the higher earthworm densities in the diverse crop rotation, in addition to the improved quality of organic matter from pea residues. We did not find differences in soil organic carbon content between the crop rotations, but this could be due to increased consumption of the previous year's residues by the larger earthworm community in the diverse crop rotation. It is, however, unlikely that including peas and oil seed rape in a crop rotation would increase the quantity of organic matter entering the soil, as those crop species have been reported to produce equal or lower biomass of residues than wheat (Soon and Arshad, 2002).

4.2. Reduction of earthworm bioturbation under intensive tillage reflects the response of anecic earthworms

We found that earthworm bioturbation, here defined as the dry weight of soil translocated via earthworm egestion per unit area and time (Taylor et al., 2019), is largely determined by tillage intensity. This is because under no tillage, the tillage sensitive anecic earthworms increase bioturbation on average by four times compared to conventional tillage, where bioturbation is solely due to the activity of endogeic earthworms. Similar results were obtained by Pelosi et al. (2017) who studied temporal dynamics in earthworm-macropores in different cropping systems using X-ray tomography. They found that in a non-ploughed living mulch cropping system, the volume and continuity of earthworm macropores was higher than in conventional and organic ploughed systems five months after ploughing, and that pore volume and continuity were correlated with anecic earthworm biomass. Unlike for anecic densities, we did not find higher bioturbation in the diverse compared to simple crop rotation under conventional tillage. The likely reason for this is that all anecic individuals collected from the conventionally tilled plots were juveniles and thus contributed less to bioturbation due to their small body size.

We decided to study tillage intensity and crop rotation effects on earthworm bioturbation, instead of e.g. earthworm biomass, because bioturbation better describes the functional importance of earthworms. Our way of calculating bioturbation allows easy quantitative estimation of the effect of earthworms on soil functioning. It tells about the quantity of soil that, in a certain amount of time, passes the earthworm gut and is then egested elsewhere improving fertility (van Groenigen et al., 2019) and changing soil aggregation (Zangerlé et al., 2011) at that location. As such, it extends the quantification of earthworm effect from physical to chemical soil properties, such as nutrient mineralization. Simultaneously, our method is a generalization, and more preciseness could be achieved by e.g. studying egestion rates of a wider variety of species and individuals in different life stages (juveniles vs adults). In addition, parameters affecting earthworm activity other than temperature should also be considered, such as soil moisture, soil compaction, and organic matter availability (Capowiez et al., 2021; Faber et al., 2017; Hoeffner et al., 2021). With these options for improvement, and by combining the method with the information that e.g. X-ray tomography can give about the effect of earthworms on soil porosity (Capowiez et al., 2015, 2014) and aggregate formation (Le Bayon et al., 2020), it would allow sophisticated comparisons of functional differences between earthworm species, and offer valuable information for modelling purposes (Meurer et al., 2020). Despite of these possibilities for methodological improvements in calculating earthworm bioturbation, our study gives valuable insight into the potential effect of different agricultural practices on earthworm functions.

4.3. Conditions for earthworms in cultivated soils can be improved in alternative ways

Anecic earthworms are often suggested to be especially important for soil structure through creation of continuous vertical macropores which improve water infiltration (Shipitalo and Le Bayon, 2004). The importance of anecic earthworms and their functions might further increase when extreme weather events like severe rains become more frequent due to climate change (Andriuzzi et al., 2015). However, the special role of anecic earthworms for soil structure has been surprisingly difficult to prove, and more evidence was recently called for by Lang and Russell (2020). In their meta-analysis, no significant effects were found of most studied earthworm species, including the well-studied anecic species *L. terrestris*, on soil porosity and bulk density. At the same time, there is evidence that endogeic earthworms can also be important for water infiltration (Capowiez et al., 2014), and both types of earthworms seem to be equally beneficial for crop growth (van Groenigen et al., 2014). Thus, we believe that the importance of endogeic earthworms in agricultural soils should not be overlooked, and that more research is needed on the functional roles of different earthworm species and ecological groups in agricultural soils. However, it is reasonable to assume that a more diverse community fulfills a greater range of functions (Tilman et al., 2014). In this view, agricultural practices that benefit earthworms with different functional roles should be favored. Based on our study, this would mean both a reduction in tillage intensity to increase anecic earthworms and a diversification of crop rotation to increase endogeic earthworms.

Our study underpins the importance of multifactorial experiments that allow examining interaction effects of different agricultural practices for agricultural research to be meaningful for farmers. Based on the outcome from such experiments, farmers are provided with a larger choice of methods for enhancing the abundance of functionally important soil organisms such as earthworms. Each agricultural field has an individual environmental context. Choices between alternative agricultural methodologies are necessary to give farmers the tools to sustainably improve soil fertility and yields according to local needs and conditions.

Data availability statement

The data that support the findings of this study are openly available in Dryad Digital Repository at https://doi.org/10.5061/dryad.3j9kd51m4 (Torppa and Taulor, 2022).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2022.104460.

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